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Resistance of Weathered Cotton Cellulose to Cellulase Action

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Increased resistance of weathered cotton cellulose to microbial breakdown has been shown to be the result of development of resistance to the action of fungal cellulases. Photochemical activity during weathering exposure transforms the cellulose into an altered substrate that prevents access of the enzymes to susceptible sites of the cellulose molecule. It is postulated that the altered substrate consists of cellulose molecules of low degrees of polymerization, with some ring openings and altered chain ends. Weathered cellulose fails to adsorb cellulases.

Cotton fabric subjected to outdoor weathering develops resistance to microbial deterioration (1, 4, 5, 12, 31). This phenomenon occurs while fabric tensile strength is being lost because of weathering. Wagner et al. (28) observed that exposure of cotton fibers to ultraviolet (UV) irradiation increased their resistance to subsequent fungal attack. Reese (21) noted that cellulose irradiated by cathode rays showed increased resistance to the action of cellulolytic fungal enzymes. The mechanisms responsible for microbial resistance that relate to weathering have been ascribed either to (i) selective deterioration of "amorphous" cellulose by photochemical action leaving "crystalline" cellulose which is more highly resistant to fungi, (ii) removal of microbial nutrients such as waxes, pectic substances, traces of salts or sugars during weathering, thereby limiting rate of growth of contaminating microorganisms, or (iii) formation of substituted or modified cellulose derivatives, through chemical contaminants or by actinic energy, resulting in substrates not readily utilized by microorganisms. The exact mechanisms, however, have not been determined. This paper reports that weathering increases the resistance of cellulose to enzymatic hydrolysis and indicates that this causes the increased microbial resistance by weathered cotton cellulose.

MATERIALS AND METHODS

Fabrics studied were a 7.2 oz bleached cotton twill exposed outdoors for periods ranging from 0 to 14 months (September, 1962 to November, 1963) for a total of 162,930 Langleys (cal/cm^2), an 8 oz blue-lined cotton duck exposed outdoors 0 to 8 months (April to December, 1960) for a total of 87,942 Langleys, and a 9.85 oz duck, thoroughly scoured and bleached, exposed outdoors 0 to 4 weeks (July, 1965) for a total of 12,308 Langleys. Cellulosic substrates used in the enzyme studies included raw cotton sliver and carboxymethyl cellulose (CMC, Hercules, Inc.), degree of substitution (DS) 0.50.

The fabrics were exposed at the Sudbury, Mass., Annex of the U.S. Army Natick Laboratories, an area free of immediate visible industrial air contamination. Samples were tacked on unshaded, open-backed wooden racks facing south at an angle of 45° from the vertical and at a minimum distance of 30 inches above the ground. Weathered panels were removed at weekly or monthly intervals, cut into 1.25 inch (3.18 cm) strips along the warp direction and ravelled to the standard 1 by 6 inch (2.54 by 15.24 cm) strips. Radiation dosage was monitored by a fixed Eppley Precision Spectral Radiometer also mounted at 45° facing south.

Soil burial tests were done with 1 by 6 inch ravelled strips in accordance with method 5762 of Federal Specification CCC-T-191b (27). Textile specimens were conditioned for at least 24 hr at 70 ± 2 F and 65 ± 2% relative humidity before tensile testing. Tensile strengths of 1 by 6 inch ravelled specimens were determined with an Instron tensile tester (model TT-C1) in accordance with method 5104.1 of Federal Specification CCC-T-191b (27). Fluidity in cuprammonium hydroxide was determined with Shirley X-type viscometers by the method used by the British Cotton Industry Research Association (8). Degree of polymerization (DP) was calculated from the equation derived by Battista (3) for 0.5% cellulose solutions: DP = 2160 [log (relative viscosity + 1) − 0.267].

The cellulase solutions used were culture filtrates of *Trichoderma viride* QM 6a, grown on mineral salts plus 1% wood cellulose (15). In the experiment on periodate oxidized fabric, an acetone (66%) precipitated cellulase was used. Enzyme activity was measured by production of reducing sugar from cotton sliver for the C_1 component of the cellulase complex, and from CMC for the C_x, $\beta 1 \rightarrow 4$ glucanase (16). For action on fabric in the initial tests, 200 mg of substrate was weighed into a test tube, wet with 0.5 ml of buffer (0.5 M acetate, *p*H 4.0) plus 4.5 ml of enzyme solution, full strength or diluted 1:70 with water.

Tubes were stoppered and incubated at 40 C without shaking. Glucose was determined after incubation by a dinitrosalicylic acid method (26). In later tests where enzyme adsorption and tensile strength loss were measured, weathered and unweathered cotton twill in strips approximately 1 by 6 inches (weight ca. 1 g) were rolled up and placed in test tubes. Each strip was wet with 0.05 M acetate buffer, *p*H 4.0 (1 ml), plus *T. viride* cellulase (9 ml) and incubated at 40 C. After 5 days, sugar and cellulase as C_1 and C_x in solution were determined. Each strip was rinsed thoroughly, damp dried between paper towels, and then conditioned and tested for tensile strength. All values were corrected for appropriate enzyme and substrate blanks. Merthiolate (Eli Lilly Co.) was added at 0.005% to all digests to prevent microbial growth.

Periodate oxidation was carried out by the method of Nevell (17), by using .015 M sodium metaperiodate. Eighteen 1 by 6 inch strips of fabric, totalling 18 g were placed in a 2,000-ml beaker to which 900 ml of reagent was added. One hundred milliliters of the reagent was placed in a 150-ml beaker as a control. Both beakers were capped with aluminum foil to prevent excessive evaporation and held in the dark at 21 C. Six strips were harvested at intervals of 6, 12, and 24 hr. Periodate concentration remaining in both beakers was determined by diluting the solutions 1:250 and reading the values at 223 nm in a Beckman DK-2A spectrophotometer (2).

Infrared data on cotton strips were obtained from KBr pellets by utilizing the dual grating Perkin-Elmer 237 infrared spectrophotometer. KBr sample preparation was performed in accordance with the procedure given by O'Connor et al. (18).

X-ray diffraction studies were carried out by techniques described by Klug and Alexander (13). Degree of crystallinity was determined by obtaining the crystalline/amorphous ratio of the measured intensities for the characteristic (002) and (101) diffraction peaks of the cotton X-ray pattern, by utilizing copper K_2 radiation.

Gas chromatographic studies were carried out with a Model 1609 flame ionization gas chromatograph (F & M Scientific Division, Hewlett-Packard Corp.). The cotton samples were hydrolyzed, and the hydrolysis components, after conversion to their trimethylsilyl derivatives, were separated on a 6 ft by ¼ inch copper column packed with 5% G.E. S.E.-30 on Anakrom S.D., 70/80 mesh, using a temperature program from 100 to 210 C at 5 C/min. The cotton fabrics were ground to 20 mesh in a Wiley mill and were either reduced with sodium borohydride or oxidized with chlorous acid prior to hydrolysis. For reduction, 150 mg of sample was suspended in 50 ml of a water solution containing 150 mg of sodium borohydride and shaken for 24 hr at 30 C. Samples were oxidized by treatment of 150 mg with 20 ml of 0.4 M chlorous acid, freshly prepared by acidification of sodium chlorite to *p*H 2.5 with glacial acetic acid. After filtration and washing, the samples were hydrolyzed overnight at room temperature with 1 ml of 72% sulfuric acid, diluted to 10 ml, and refluxed for 2 hr. The solution was neutralized with an ion-exchange resin, and then evaporated at 50 C under reduced pressure to a yellow sirup. The components of this residue were converted to their trimethylsilyl derivatives to provide suitable volatility for gas chromatography. The trimethylsilyl derivatives were prepared by the procedure of Brobst and Lott (7) by dissolving the residue in 1 ml of pyridine and then adding 1 ml of hexamethyldisilazane and 0.1 ml of trifluoracetic acid. Five microliters of this solution was used for the chromatography.

RESULTS

Effects of weathering of cotton on its susceptibility to fungal and enzyme degradation. As the cotton twill and duck fabrics were weathered, tensile strength, cuprammonium fluidity, and radiation measurements were taken at periodic intervals. The fabrics, as expected, showed the typical drop in tensile strength and increase in fluidity with continued outdoor exposure (Fig. 1), reflecting the effects of actinic energy on the cellulose. These fabrics, after weathering, also showed the expected greater resistance to soil burial as compared to unweathered fabric (Fig. 2).

Weathered cotton fabrics were markedly resistant to hydrolysis by cellulase (Tables 1, 2). The unweathered fabrics were susceptible to the *T. viride* cellulase even at 0.1 dilution of enzyme. The susceptibility to cellulase falls markedly during the first and second months of weathering and then levels off, showing little further change even after 14 months. The resistance to microbial breakdown as shown with soil burial is, therefore,

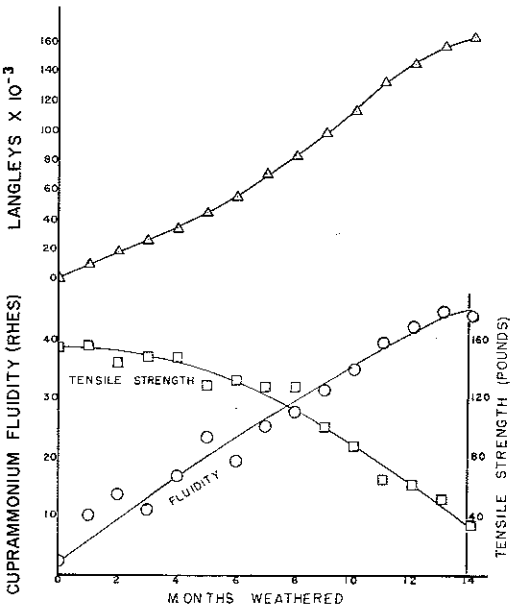

FIG. 1. *Effect of weathering on tensile strength and fluidity of cotton twill.*

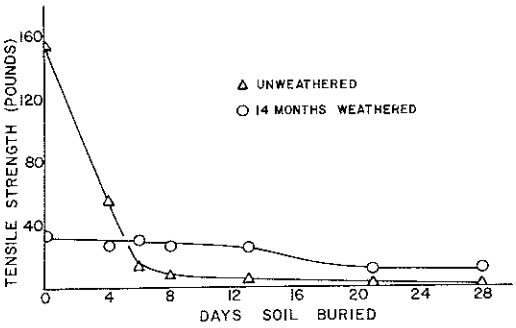

FIG. 2. *Effect of weathering of cotton twill on subsequent loss of tensile strength during soil burial.*

TABLE 1. *Effect of weathering on activity of cellulase on cotton twill*[a]

Time weathered	Glucose produced (mg/ml) with enzyme full strength		Glucose produced (mg/ml) with enzyme 1/10 strength	
	1 day	4 days	1 day	4 days
months				
0	3.07	5.76	1.12	1.76
1	1.10	3.11	0.60	1.04
3	0.94	1.74	0.50	0.96
3	0.80	1.54	0.40	0.80
4	0.80	1.54	0.44	0.76
5	0.62	1.06	0.32	0.60
6	0.64	1.24	0.34	0.68
7	0.56	0.97	0.28	0.52
8	0.48	0.90	0.30	0.58
9	0.50	1.10	0.34	0.65
10	0.48	0.90	0.30	0.58
11	0.48	0.94	0.32	0.65
12	0.48	0.94	0.32	0.59
13	0.54	0.90	0.38	0.66
14	0.52	0.90	0.38	0.70

[a] Two hundred milligrams of fabric (unground), 0.5 ml of 0.5 M acetate buffer (*p*H 4.0), and 4.5 ml of *Trichoderma viride* culture filtrate containing 0.005% Merthiolate, incubated, unshaken, at 40 C.

confirmed by the enzyme study. The theory that lack of microbial nutrients on weathered material is responsible for resistance does not appear to be valid because the unweathered fabrics contained no starch, and the presence or absence of other noncellulosic "microbial nutrients" per se would have no influence on the rate of the enzyme hydrolysis. To be quite certain of this point, an extremely well scoured and bleached 9.85 oz cotton duck was chosen for further study. The nature of the scouring and bleaching process was sufficient to remove any starch sizing, hemicelluloses, pectins, and other extraneous noncellulosic

TABLE 2. *Activity of Trichoderma viride cellulase on cotton duck before and after weathering*[a]

Time weathered	Glucose produced (mg/ml) by enzyme action	
	1 day	6 days
months		
0	2.13	6.51
2	0.81	2.46
4	0.69	2.21
6	0.65	2.06
8	0.59	1.96

[a] Two hundred milligrams of fabric (unground), 0.5 ml of 0.5 M acetate buffer (*p*H 4.0), and 4.5 ml of *T. viride* culture filtrate containing 0.005% Merthiolate, incubated unshaken at 40 C.

materials. The fabric was weathered in the usual manner and harvested at weekly intervals for 1 month, after which the enzyme digestion was run. At 6 days, the fabric was removed from the enzyme solution, washed in water and alcohol, dried at 80 C, and weighed. The results (Table 3) are in good agreement with, and show the same decline in susceptibility as, the other cotton fabrics.

No significant weight changes were found in the cotton twill during weathering. However, an interpretation of weight data cannot be made since minor losses in weight would be offset by increases due to shrinkage and the accumulation of dirt. The degree of crystallinity as determined by X-ray diffraction was 61% before weathering, 59% after one month of exposure, 64% after 7 months, and 64% after 14 months. These changes are not significant. It thus appears improbable that resistance is due to the selective removal or hydrolysis of components of the cellulose, e.g. "amorphous" cellulose.

There was a possibility that the resistance of the weathered fabric was due to the presence of an enzyme-inactivating factor produced during weathering. We, therefore, incubated 1 by 6 inch (1 g) strips of the cotton twill with 10 ml of enzyme for 5 days and determined reducing sugar produced, percent loss in tensile strength, and enzyme remaining in solution. As before, the unweathered fabric was the most susceptible to enzyme action with greatest sugar production and greatest per cent loss in tensile strength, but this sample had the least cellulase remaining in the supernatant (Table 4). The weathered samples were less susceptible to enzyme action, and more of the cellulase was found in solution. For the 14-month-weathered sample, all of the C_x and C_1 were found in the supernatant. Obviously, weathering did not produce enzyme-

TABLE 3. *Activity of Trichoderma viride cellulase on scoured cotton before and after weathering*[a]

Time weathered	Glucose produced (mg) by enzyme action		Weight loss (mg) at 6 days
	1 day	6 days	
weeks			
0	9.75	31.5	27
1	6.45	22.0	23
2	4.95	19.0	19
3	5.05	17.0	11
4	3.95	17.5	17

[a] Two hundred milligrams of fabric (unground), 0.5 ml of 0.5 M acetate buffer (*p*H 4.0), and 4.5 ml of *T. viride* culture filtrate containing 0.005% Merthiolate, incubated unshaken at 40 C.

TABLE 4. *Effect of weathering of cotton twill on cellulase activity and on adsorption of cellulase*[a]

Time weathered	Glucose produced (mg/ml) by enzyme action		Enzyme in solution (units/ml) at 5 days		Breaking strength of fabric (lb)		
	1 day	5 days	C_X	C_1	Initial	5 days	Loss
months							%
0	4.8	10.0	82	6	154	103	33
1	2.8	5.9	210	11	155	142	8
2	2.4	4.8	240	12	144	132	8
7	1.5	2.9	330	17	127	123	3
14	1.6	3.2	440	24	33	33	0

[a] One-gram (1 by 6 inch) strip of fabric, plus 1 ml of 0.5 M acetate buffer (*p*H 4.0), and 9.0 ml of *T. viride* culture filtrate containing 0.005% Merthiolate, incubated unshaken at 40 C.

inactivating substances. This study established that cellulase is strongly adsorbed by unexposed fabric and that adsorption decreases on weathering. It suggested that changes take place during weathering of cellulose that are responsible for imparting resistance to enzyme adsorption and to subsequent enzymatic breakdown.

Physical and chemical changes in cotton resulting from weathering and from periodate oxidation. An extended study of grey cotton duck exposed to weathering, carried out by Yelland (31), revealed an alkaline sensitivity, a chain breakage caused by alkali, in the weathered fabric similar to that observed in cellulose oxidized with periodate. Both weathered and oxidized cellulose showed increased resistance to soil burial. Since sensitivity to alkali is an indication of oxidation on carbons 2 and 3 of the anhydroglucose unit of the cellulose molecule, it is reasonable to assume that oxidation at this point also might be involved in preventing enzymatic breakdown

TABLE 5. *Uptake of periodate from solution during oxidation of cotton twill*

Time oxidized	Periodate uptake	Oxidation
hr	*g/strip*	%
0	0	0
6	0.0250	1.9
12	0.0475	3.6
24	0.0707	5.4

TABLE 6. *Activity of Trichoderma viride cellulase on cotton twill before and after periodate oxidation*[a]

Periodate oxidation	Glucose (mg/ml) produced by enzyme action		Cellulase (units/ml) in solution (4 days)	
	1 day	4 days	C_X	C_1
hr				
0	3.8	7.6	93	9.4
6	1.5	4.1	99	10.0
12	1.4	3.8		
24	1.4	3.4	97	11.8

[a] One-gram (1 by 6 inch) strip of fabric plus 10 ml of acetone-precipitated *T. viride* cellulase at 2 mg/ml in 0.05 M acetate buffer (*p*H 4.0) containing 0.005% Merthiolate, incubated unshaken at 40 C.

of the substrate. A study of enzyme activity on cellulose oxidized with periodate was, therefore, undertaken to determine the involvement of oxidation on carbons 2 and 3 and the relationship, if any, of this oxidation to enzyme adsorption.

Cotton twill was oxidized in 0.015 M sodium metaperiodate, as described earlier, for periods of 6, 12, and 24 hr. The degree of oxidation expressed as moles of periodate per glucose unit is shown in Table 5, the consumption of 1 M periodate corresponding to 100% oxidation. Enzyme treatment was carried out with acetone-precipitated *T. viride* cellulase at 2 mg/ml in 0.10 M acetate buffer, *p*H 4.0. One by six inch strips of fabric were incubated at 40 C with 1 ml of enzyme solution per 100 mg of fabric.

Periodate-oxidized fabric showed increased resistance to enzymatic hydrolysis (Table 6) similar to, but of lesser magnitude than, that found for weathered fabric, However, in contrast to the effect of weathering, periodate oxidation had no significant effect on adsorption of cellulase by cellulose (Fig. 3, 4). It is thus apparent that oxidation on the carbons 2 and 3 may play a role in blocking enzymatic activity but that this oxidation per se is not involved in blocking enzyme adsorption by the substrate.

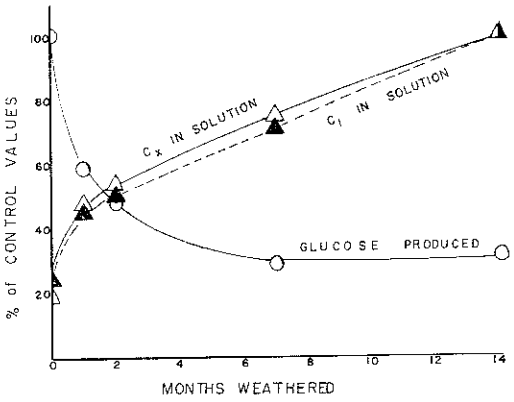

FIG. 3. *Glucose and enzyme concentration in solution after weathering of cotton fabric. Symbols:* △, C_x *in solution (100 = original enzyme concentration);* ▲, C_1 *in solution (100 = original enzyme concentration);* ○, *glucose produced by enzyme action (100 = amount produced from unweathered cellulose).*

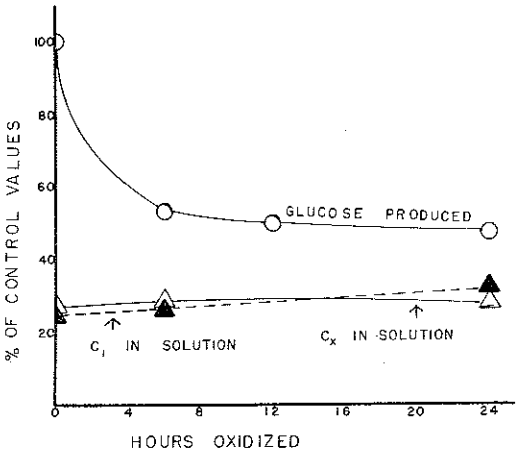

FIG. 4. *Glucose and enzyme concentration in solution after periodate oxidation of cotton fabric. Symbols:* △, C_x *in solution (100 = original enzyme concentration);* ▲, C_1 *in solution (100 = original enzyme concentration);* ○, *glucose produced by enzyme action (100 = amount produced from unweathered cellulose).*

Infrared absorption spectra of the weathered and unweathered cotton showed only the normal spectrum of cellulose, even after 14 months of weathering. Spectrograms of periodate-oxidized fabric contained a weak, barely perceptible band at 1,730 cm^{-1} which, considering the intensity of the C=O stretching band, would correspond to a very low concentration of free carbonyl. However, in periodate-oxidized cellulose, an inverse relationship has been demonstrated between the intensity of the C=O band and the moisture content of the material. Studies made by Spedding (24) indicate that about 75% of the aldehyde groups are in the form of hemialdals, the rest being free hydrated aldehydes or hemiacetals. Thus a small amount of oxidation would not be detected spectrophotometrically.

During work on the structural analysis of methylolmelamine-treated cotton, a procedure was developed for determining by gas-liquid chromatography the extent of substitution on the hydroxyl groups of the cellulose (29). The cotton was oxidized with periodate to open up the resin-bound polymer network and make free hydroxyl groups more accessible to blocking agents. After total hydrolysis, unsubstituted anhydroglucose units, as a result of the periodate treatment, were recovered as erythritol.

This procedure was adapted to the analysis of the weathered and periodate-oxidized fabrics. The relative amounts of erythritol derived from each material after borohydride reduction and hydrolysis are shown in Fig. 5. While the amount obtained was considerably less from the weathered than from the periodate-oxidized fabric, the presence of erythritol showed that very little oxidation had taken place on the anhydroglucose units during the weathering process. It was not certain that this oxidation was confined to carbon atoms 2 and 3, as with periodate oxidation, or whether carbon atom 6 was involved.

The use of an oxidant to convert further the aldehyde groups to carboxyl groups prior to hydrolysis afforded a means of distinguishing among the three sites of oxidation on the anhydroglucose units. With oxidation only on carbon atom 6, there would be no ring cleavage, and the product would be glucuronic acid. With oxidation only on carbon atoms 2 and 3, the product would be erythronic acid. Finally, oxidation on all three carbons would yield a four-carbon fragment with two carboxyl groups, namely tartaric acid.

The weathered cotton was subjected to oxidation with chlorous acid by the procedure of Rutherford et al. (22). The chromatogram obtained is shown in Fig. 6. Of the three possible hydrolysis products, only erythronic acid was observed. Through the intervals where the products are expected, the sensitivity was increased by a factor of 8. A slight hump in the baseline at the retention of tartaric acid was present in all samples, including unweathered and borohydride-reduced samples. No glucuronic acid was observed although the instrument response was such that 0.5 μg, corresponding to approximately 0.02%, would be detectable. It would appear, therefore, that oxidation on the anhydroglucose units of the weathered fabric is limited to carbon atoms 2 and 3 with no involvement at carbon

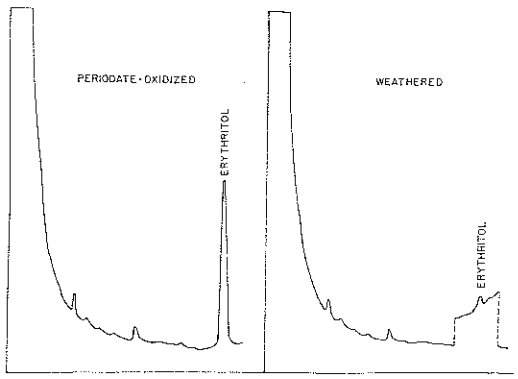

FIG. 5. *Chromatogram of erythritol from periodate-oxidized and weathered cotton. Sensitivity increased eight times through interval between dotted lines.*

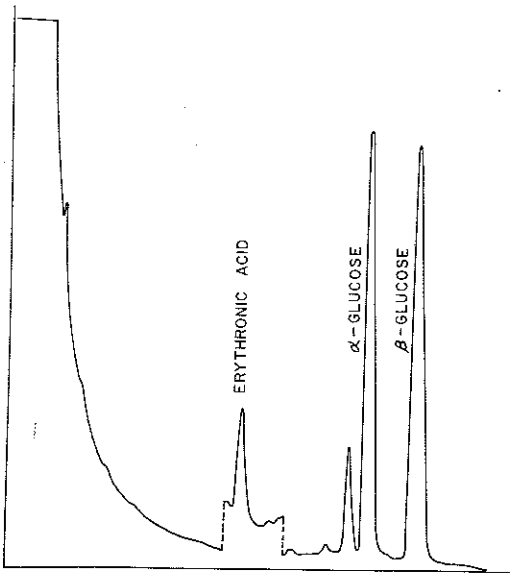

FIG. 6. *Chromatogram of hydrolysis products from weathered cotton oxidized with chlorous acid. Sensitivity increased eight times through interval between dotted lines.*

atom 6, as evidenced by the absence of significant amounts of tartaric acid and glucuronic acid in the hydrolysis product.

Changes other than substitution on the hydroxyl groups are not revealed by the gas chromatographic procedure because of the total hydrolysis step. To obtain additional information, DP values were calculated from the fluidity values. It was considered that the increase in fluidity caused by the action of cuprammonium solution on oxidized glucose units would be minor, based upon the gas chromatographic results and upon the work of Yelland (31). A

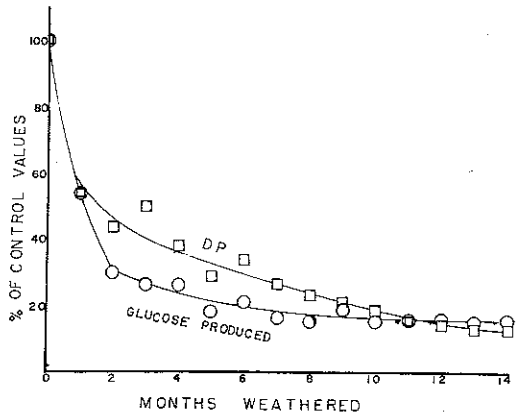

FIG. 7. *Enzyme activity and degree of polymerization after weathering of cotton twill.*

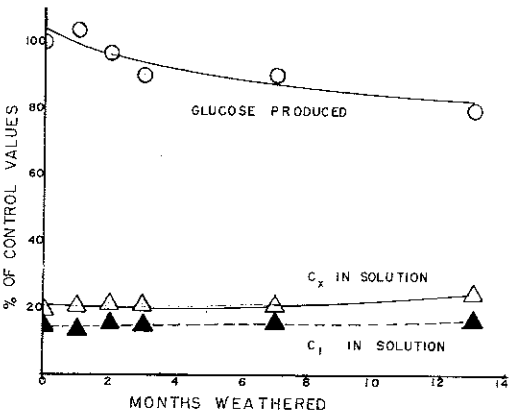

FIG. 8. *Glucose and enzyme concentration in solution after ball milling of weathered cotton twill. Symbols: ○, glucose produced by enzyme action (100 = amount produced from unweathered cellulose); △, C_x in solution (100 = original enzyme concentration); ▲, C_1 in solution (100 = original enzyme concentration).*

striking similarity was observed between the change in DP upon weathering and the enzyme activity, in that the greatest reduction in both took place during the first two months of exposure, with the rate levelling off thereafter (Fig. 7). Decreased chain length per se, however, would not be expected to bring about an increase in resistance and could be coincidental with the appearance of resistance to enzyme activity. Resistance at this point, therefore, could be attributed solely to photoxidation of carbon atoms 2 and 3.

A factor that must be considered, however, is the difference in enzyme adsorption between periodate-oxidized and weathered cotton. Since periodate oxidation of cellulose does not result

TABLE 7. *Degree of polymerization of cotton twill before and after ball milling*

Time weathered	Degree of polymerization			Reduction after ball milling
	Fabric	Wiley milled	Ball milled	
months				%
0	2,032	1,848	1,307	36
1	1,132	1,037	878	22
2	1,063	981	815	23
3	1,056	1,037	730	31
7	738	732	592	20
13	434	403	348	20

in significant change in DP (22) and there is adsorption of enzyme in periodate-oxidized cotton, and since there is little enzyme adsorption with weathered cotton, a marked change in DP, and only a small oxidative change on the secondary carbons, the resistance of weathered cellulose thus might be due to structural alteration rather than chemical substitution. It was of interest therefore to determine whether the resistance would be affected by a change in fiber structure.

Samples from several exposure periods and also an unweathered control sample were ground to 20 mesh in a Wiley mill and subjected to 24 hr of ball milling. The ground samples and some of the original fabric were exposed to *T. viride* cellulase. The result for unground fabric was as before, and similar results were found after Wiley milling, although the weathered samples showed some increase in enzyme susceptibility. For ball milled fabrics, however, there was a large increase in enzyme activity for all samples, including the unweathered control, and the resistance due to weathering practically disappeared (Fig. 3 versus Fig. 8). DP was calculated for each sample (Table 7). Although the ball mill reduced all samples to a fine powder, a uniform chain length did not result but instead varied according to the initial DP.

DISCUSSION

The action of weathering on cotton textiles includes the aggregate effects of sun, air, moisture, temperature, wind, and particulate matter, the effect of sunlight being regarded as the most significant of these factors. Numerous studies have been made of the photolytic degradation of cellulose by UV light. After UV irradiation of cotton in an inert atmosphere, degradation results upon exposure to oxygen, the effect being a decrease in degree of polymerization, an increase in aldehyde and carboxyl content, and liberation of CO and CO_2. This effect was reported by Stillings and Van Nostrand (25) who found that it could be produced or deferred at will by alternating between the two atmospheres. If the exposure time in oxygen was sufficiently long, the postirradiation effect came to an end. Heuser and Chamberlin (11) obtained the postirradiation effect with cellulose triacetate, indicating that the hydroxyl groups are not involved in the reaction which leaves the glycosidic linkages unstable to oxygen. In both of these studies, the UV source was heterogenous with respect to wavelength. Launer and Wilson (14) established differing effects for different UV regions. With monochromatic light at 254 nm, water was found to retard and oxygen to have no effect on the degradation of cotton and wood cellulose. The reverse was found to be true for the near UV and visible range from 330 to 750 nm. The evolution of hydrogen, previously unreported, was observed by Flynn et al. (9) upon irradiation of cellulose at 254 nm in vacuo. The hydrogen production was not related to chain scissions or to aldehyde and carboxyl groups initially present and was attributed to the photolysis of alcohol groups.

Beelik and Hamilton (6) studied the water-soluble fraction obtained from UV irradiation of wood cellulose. They found that about 60% of the products were neutral sugars, including glucose, arabinose, xylose and oligosaccharides containing these sugars as terminal reducing units. Volatile and nonvolatile acids were also formed. In a study of the water-soluble products from irradiation of cotton cellulose by Gingras et al. (10), glucose, arabinose, cellobiose and β-D-glucosido-D-arabinose were identified. There was some indication of the presence of glycollic acid and erythrose, but these compounds were not consistently present. The authors concluded that the primary photolytic action is the cleavage of glucosidic bonds with initiation of photolysis in the vicinity of carbon atoms 1 and that oxidation of the secondary alcohol groups plays a minor part in the overall process. Williams (30) recently presented data from the irradiation of cellulose in cadoxen solution indicating that the primary photolytic scissions are distributed between the glucosidic bridge and the C_1–C_2 bonds. These results are in accordance with the work of Beelik and Hamilton who found D-glucose and D-arabinose residues.

Previous studies on the weathering of cotton by Race (19), Yelland (31), and Abrams (1) show effects similar to those resulting from photochemical degradation, namely shortening of the cellulose chains as evidenced by increased fluidity or decrease in tensile strength, or both,

and by an increase in reducing power and carboxyl groups. In the latter two studies, resistance to microbiological attack was reported. Yelland (31) postulated that oxidation on the anhydroglucose units was the mechanism of cellulose degradation (concomitant with decrease in chain length) and, by implication, of increase in mildew resistance which was found in soil burial. Abrams (1) suggested that the amorphous component of cellulose was deteriorated rapidly by photochemical action, leaving the crystalline portion resistant to the action of *Chaetomium globosum* on agar culture medium.

The results of the present study show that outdoor weathering of cotton leads to a marked decline in susceptibility to hydrolysis by fungal cellulases. Sufficient information on which to base a definite conclusion as to mechanism has not been obtained, but certain conclusions and suppositions can be drawn. The gas chromatographic analyses show that some oxidation took place with resultant splitting of the C_2–C_3 bond. Although the amount was small, it may be significant if concentrated on the surface of the fibers. There is a difference, however, in the enzyme activity on weathered and periodate-oxidized cotton. Enzyme activity was reduced by 50 to 60% on the periodate-treated fabric with a 5% degree of oxidation, but was reduced by 70 to 85% on the weathered material, with a degree of oxidation estimated to be less than 0.1%. Moreover, the periodate-oxidized fabric adsorbed enzyme in the same amount as the unoxidized unweathered control (about 75%), whereas the adsorption on the weathered samples was greatly diminished. Obviously, factors other than oxidation at the C_2–C_3 bonds per se must be involved.

The most conspicuous change in the weathered cloth was the reduction in degree of polymerization, most of which took place during the first 2 months. The DP values are averages representing molecular weights which may vary widely within a sample. The reaction must commence on the surface layers of the fiber and advance inwardly. As it proceeds, the outer-reacted layers apparently exert a screening effect to suppress the photodegradation. The similarity of the enzyme activity curve to the DP curve with time of weathering suggests that change in DP may be related to the development of resistance to enzyme activity. Results from the ball-milling indicate that resistance to cellulolytic enzymes may be structural or, more precisely, topographical.

Since the X-ray diffraction studies revealed no significant differences between weathered and unweathered samples, any changes in the cellulose must affect only a small percentage of the glucose residues. At least two adjacent, unaltered glucose units are required for cellulase action (20). In a soluble derivative, a degree of substitution of 0.5, uniformly distributed, would be required for complete resistance to cellulase. In the case of solid cellulose, however, many bonds are unavailable because of structural inaccessibility and hydrogen bonding. Therefore, a small number of altered glucose units at fiber surfaces and chain ends might confer a substantial amount of resistance to enzyme action. As the chain lengths are shortened by photochemical activity during weathering, there is an increase in altered chain ends along with the development of oxidized secondary carbons. In this situation, it is conceivable that cellulose molecules of low DP with some ring openings, and with terminal pentose residues or terminal carboxyl groups, would be of such configuration that cellulases would be prevented from approaching susceptible sites on the molecule. If the premises are accepted that (i) photochemical changes take place preferentially in the less ordered areas of cellulose, (ii) that the highly ordered areas of cellulose are relatively resistant to enzyme activity, (iii) that highly ordered areas may comprise from 50 to 95% of the cellulose matrix and (iv) that the altered amorphous cellulose is at or near the surface, then the idea is not far fetched that a very small oxidative change along with a change in DP in the affected cellulose molecules can block enzyme access to a susceptible site. It appears to us that this concept is in consonance with the recent thoughts of Selby (23), who feels that there are relatively few susceptible sites in the highly ordered cellulose chains, that enzyme attack is highly localized, and that prevention of biodeterioration at low levels of substitution in the cellulose chain is feasible.

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